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Stages of Larval Development and Stem Cell Population Changes During Metamorphosis of a Hydrozoan Planula

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Abstract. Scanning electron microscopy and light histology were used to reveal the changes in overall morphology and in stem cell differentiation and distribution that occur as a free-swimming, solid hydrozoan planula larva is transformed into a sessile, hollow adult polyp. Eight stages of development are described: young 10-hour planula, mature 48-hour planula, attaching planula, disc, pawn, crown, immature polyp, and primary adult polyp. The larval interstitial stem cell population (interstitial cells, nematocytes, ganglion cells) undergoes dramatic changes during metamorphosis: (1) distribution patterns change, (2) certain larval derivatives disappear, (3) new types of derivatives differentiate, and (4) migration patterns become more complex. This study is the first to examine how a stem cell system develops in an organism that goes from embryo to larva to adult.

Introduction

The stem cell, found in metazoans from sponges to vertebrates, is an intriguing but little understood cell type. Stem cells, by definition, are not terminally differentiated: they have the ability to divide, not only generating more stem cells (self renewal) but also yielding a variety of differentiated cell types. The mechanisms by which stem cells differentiate into different phenotypes and arrive at the appropriate location are some of the most important questions in developmental biology and metazoan evolution. Three major migratory stem cell systems have been extensively studied: vertebrate neural crest cells, vertebrate hematopoietic cells, and invertebrate cnidarian interstitial cells (Bode and David, 1978; LeDouarin, 1979; Heimfeld and Bode, 1986; Martin and Archer, 1986; Lumsden, 1988; Bronner-Fraser and Fraser, 1988; Potten and Loeffler, 1990; Weston, 1991; Martin, 1991; Medvinsky *et al.*, 1993).

The study of stem cells in evolutionarily primitive metazoans may reveal some of the earliest mechanisms used for setting up patterns of cell distribution and overall morphology. Certain marine cnidarians have characteristics that make them ideal for such a study. Embryogenesis and metamorphosis are relatively rapid and, more importantly, both embryos and adults contain a population of migratory stem cells, making it possible to compare stem cell behavior during embryogenesis with behavior in the adult.

To date, studies on cnidarian metamorphosis are few and virtually nothing is known about the behavior of the interstitial cell system during metamorphosis (Martin *et al.*, 1983; Berking, 1984; Thomas *et al.*, 1987; Weis and Buss, 1987; Plickert *et al.*, 1988; Schwoerer-Böhning *et al.*, 1990; Sommer, 1990). Thus it is not known how the interstitial stem cell lineage develops in an organism that goes from an embryo to a larva to an adult.

The embryonic interstitial cell system of the marine hydrozoan *Pennaria tiarella* has been characterized by Martin and associates (Martin and Thomas, 1977, 1980, 1981a, b; Martin and Archer, 1986; Martin, 1988a, 1990, 1991). Embryos possess a well-defined population of migratory interstitial stem cells that either divide to replenish the population or differentiate into ganglion cells or nematocytes. The adult stem cell system of this hydrozoan has also been examined, though in less detail (Martin, 1988b). Because embryos and adults of *Pennaria tiarella* are easy to obtain and manipulate, develop quickly, and are small and transparent, the interstitial stem cell system can be examined continuously starting from the moment it arises in the embryo, progressing

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through embryogenesis and formation and metamorphosis of the planula larva, and continuing in the adult.

In this study we used both scanning electron microscopy and light histology to examine the changes in overall morphology and in stem cell differentiation and distribution that occur as the free-swimming, solid planula larva of *Pennaria tiarella* is transformed into the sessile, hollow adult polyp. Our results show that the larval interstitial cell system is extensively modified during metamorphosis to produce the adult pattern.

Materials and Methods

Culture of Pennaria *adults and embryos*

Mature colonies of *Pennaria tiarella* were collected from pier pilings in Wilmington and Morehead City, North Carolina. Fronds from male and female colonies were mixed together in large finger bowls of filtered seawater; these bowls were placed in the dark at 1800 hours, and returned to the light at 2100 hours. Within 1 h after exposure to light, early cleavage stages were observed in the bottoms of the dishes. These embryos were transferred to small dishes of filtered seawater and reared at 23°C to the desired planular stage.

Forty-eight-hour planulae were incubated in cesium chloride to initiate metamorphosis (Archer and Thomas, 1983). A stock solution containing 9.76 g CsCl/100 ml distilled water was mixed with Millipore-filtered seawater at a ratio of 1 (stock):10 (seawater). Planulae were placed in 4 ml of this mixture for 3 h; some of these animals were fixed for scanning electron microscopy (SEM) immediately after the cesium treatment. Planulae placed in cesium for 3 h were also returned to dishes of Millipore-filtered seawater and allowed to continue their development. Treated planulae were fixed for SEM after a recovery period of 2 (disc stage), 6 (pawn stage), 12 (crown stage), 14 (immature polyp), or 18 (primary polyp) h.

Scanning electron microscopy (SEM)

For SEM, 10- and 48-h control planulae, cesium-treated planulae immediately after treatment, and cesium-treated planulae allowed to recover for various times were fixed for 1 h in 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer, pH 7.4. Samples were rinsed three times in the phosphate buffer, postfixed for 1 h in 2% osmium tetroxide in 1.25% sodium bicarbonate buffer, pH 7.2, then rinsed three times in the sodium bicarbonate buffer. Animals were dehydrated through a graded series of ethanols to 100%, critical-point dried using CO_2, mounted on metal stubs, and sputter coated with gold palladium for 1 min in a Denton sputter coater. Samples were viewed and photographed with a JEOL JSM T-300 scanning electron microscope operated at 25 kV.

Light microscopy

Control planulae (various ages) and stages of metamorphosis comparable to those processed for SEM were fixed for 1 h in 10% formalin in seawater. Samples were dehydrated for 15 min each through an ascending alcohol series (25%–100% ethanol), followed by a 20-min rinse in 100% ethanol: 100% tertiary butyl alcohol (1:1), and an overnight incubation in 100% tertiary butyl alcohol. After being infiltrated and embedded in Paraplast Plus paraffin, animals were serially sectioned at 8 μm. The sections were mounted on glass slides and stained with azure B, which specifically stains the interstitial cells and their derivatives (Martin, 1991). Slides were viewed and photographed using a Zeiss standard research microscope.

Results

Planular morphology and stages of metamorphosis: General observations

Between 8 and 10 h postfertilization the gastrula of *Pennaria tiarella* elongates in an anterior-posterior direction to produce a fat, ciliated, free-swimming planula larva (Fig. 1). This 10-h larva has a distinct enlarged anterior apical pole and a narrower posterior basal pole. The surface cells are numerous, small, and uniform in size. By 24 h postfertilization 10-h planulae narrow and elongate to form mature, metamorphosis-competent planulae. Although planula larvae are competent to metamorphose at 24 h, as shown by induction with cesium chloride (Archer and Thomas, 1983), many planulae swim in the water column for 2 to 3 days before attaching and metamorphosing. During this swimming period the larvae continue to elongate. The 48-h hydrozoan planula is solid, elliptical, and moves in the water column with its enlarged apical end directed forward (Fig. 2).

During metamorphosis the solid, nonfeeding, motile planula is transformed into a hollow, sessile, feeding adult polyp (Figs. 3–9); this process takes about 18–20 h. The apical end of the planula forms the base of the polyp, the middle region forms the stalk, and the basal end forms the hypostome and tentacles. As planulae metamorphose their morphology changes dramatically, in distinct stages known as shortening planula (Fig. 3), disc (Fig. 4), pawn (Fig. 5), crown (Fig. 6), immature polyp (Fig. 7), and primary polyp (Figs. 8 and 9).

Mature planulae of *Pennaria* attach to substrates, usually pier pilings in the wild, *via* their anterior, apical poles; shortly thereafter metamorphosis begins.

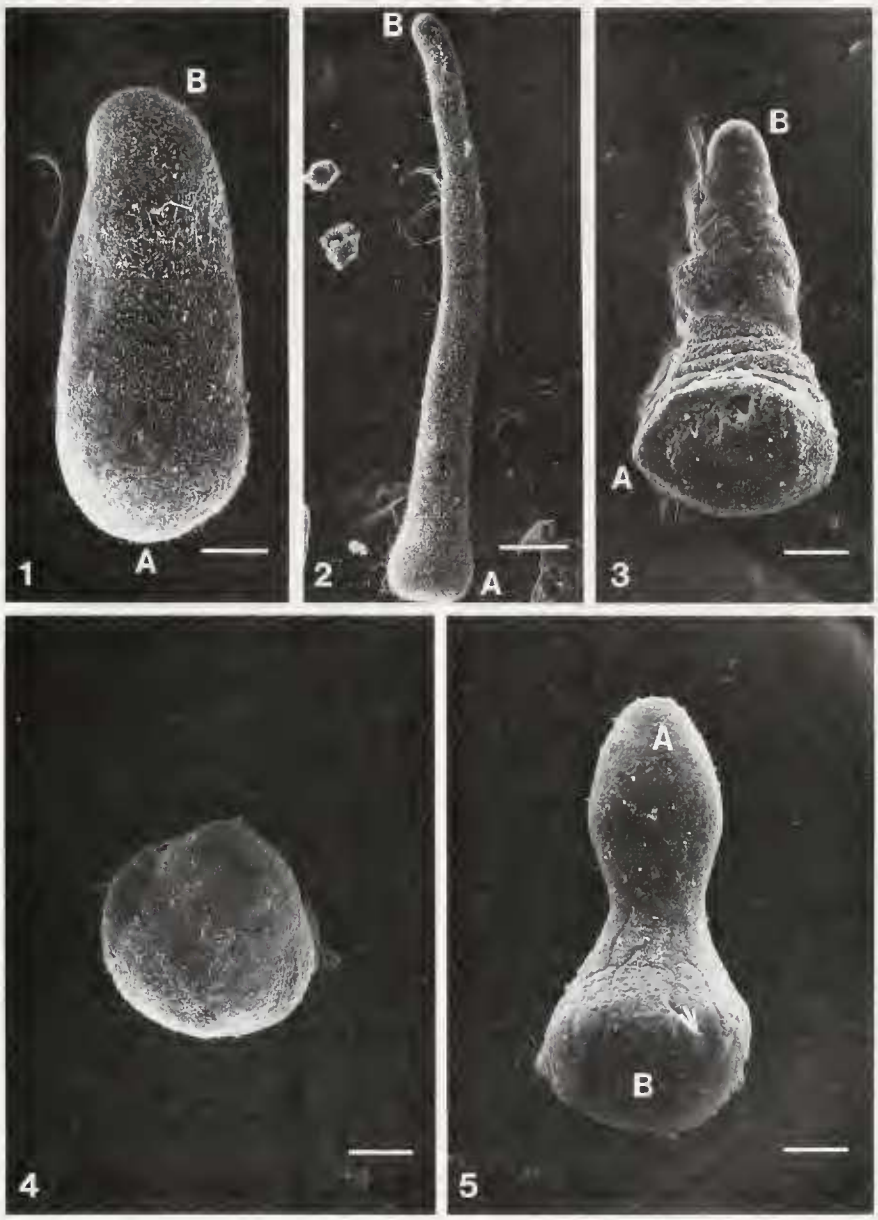


Figure 1. Ten-hour planula. The young larva is 350 μm long and 170 μm wide. A, apical end; B, basal end. Bar = 50 μm.

Figure 2. Forty-eight-hour pre-metamorphic planula. The ciliated larva moves with its enlarged apical end (A) directed forward. It is 900 μm long, 130 μm wide in the apical region, 70 μm wide in the mid area, and 50 μm wide at the basal end. B, basal. Bar = 100 μm.

Figure 3. Attached metamorphosing planula. The original anterior, apical end (A) of the larva attaches to the substrate and flattens over it while the original basal end (B) contracts towards the attached end. Thus the animal becomes short and fat, measuring 320 μm long, 160 μm wide in the apex, 93 μm wide in the middle, and 45 μm wide in the basal region. Bar = 50 μm.

Figure 4. Disc stage. The disc is a flattened ball, 180 μm in diameter, on the substrate. Bar = 50 μm.

Figure 5. Pawn stage. The base (B) of the pawn, now considered the posterior end of the animal, arises from the apical end of the planula. The anterior, apical region (A) of the pawn, derived from the basal region of the planula, forms the head and tentacles of the primary polyp. Small amounts of perisarc material (arrow) are deposited at the base. The pawn is 350 μm tall, 160 μm wide in the anterior head, 73 μm wide in the mid-stalk, and 106 μm wide at the posterior base. Bar = 50 μm.

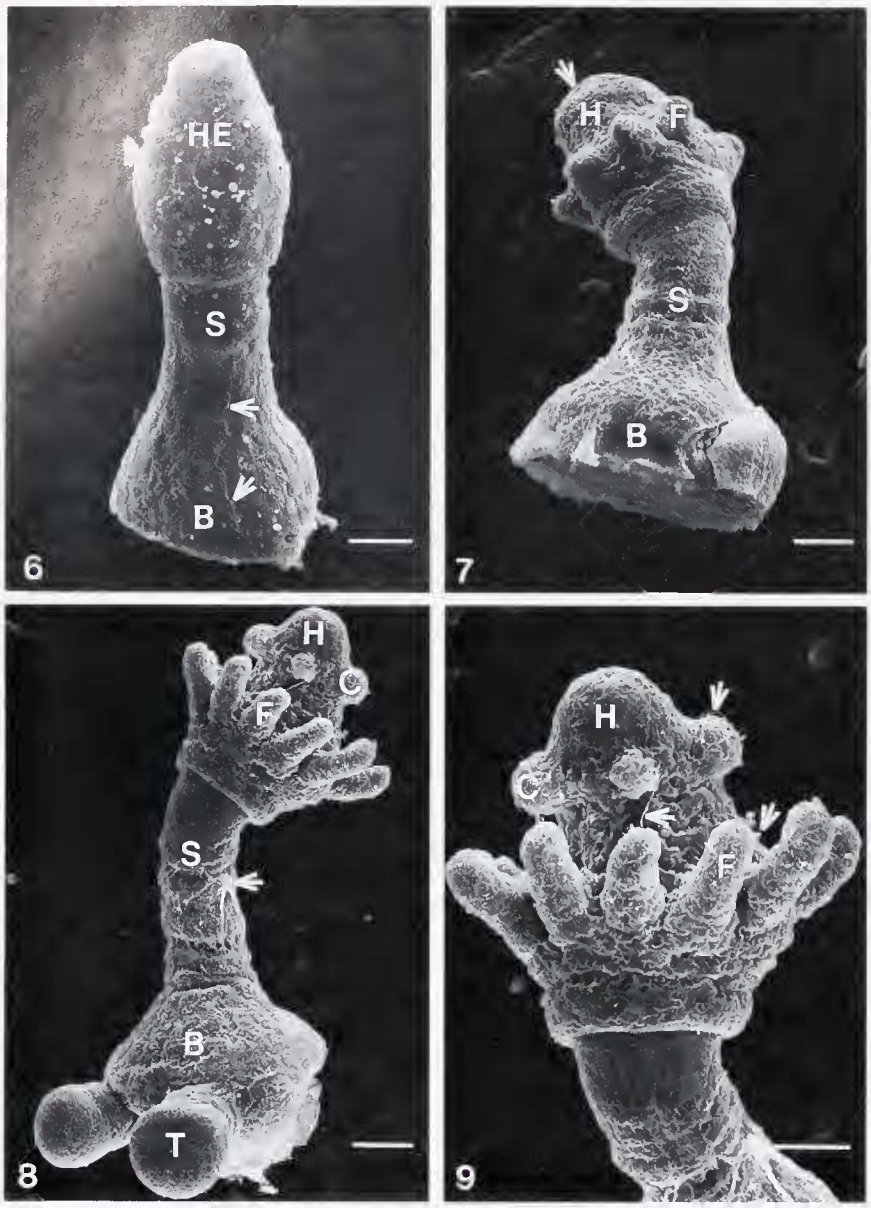


Figure 6. Crown stage. A distinct head region (HE), stalk (S), and base (B) are evident. Perisarc material (arrows) covers the surface. The crown is 427 μm tall, 125 μm wide in the apical crown region, 80 μm wide in the stalk region, and 166 μm wide at the base. Bar = 50 μm.

Figure 7. Immature polyp. The head region consists of a conical mound, the hypostome (H), a mouth (arrow), and a ring of forming filiform tentacles (F). A stalk (S) connects the head to the base (B). The polyp is 340 μm tall, 120 μm wide in the anterior hypostome region, 73 μm wide in the mid-stalk area, and 206 μm wide in the posterior base. Bar = 50 μm.

Figure 8. Primary polyp. The head is composed of the hypostome (H), short capitate tentacles (C), and the longer filiform tentacles (F). A narrow stalk (S), covered by perisarc (arrow), extends from the head to the base (B) of the adult. Stolons (T) emerge from the base. The primary polyp is 500 μm tall, 200 μm wide in the crown area, 70 μm wide in the mid-stalk area, and 200 μm wide in the basal area. Bar = 50 μm.

Figure 9. Enlarged head region of a primary polyp showing hypostome (H) with capitate (C) and filiform (F) tentacles. These tentacles are armed with nematocytes (arrows). Bar = 50 μm.

Shortening planula (Fig. 3). Once attached the apical planula end flattens and expands over the substrate while the basal end contracts down towards the expanded apical pole. Thus the attached larva becomes short and fat.

Disc (Fig. 4). Within 2 h of attachment the basal end of the larva has completely contracted and a round disc shape is formed. The animal appears as a small, flattened ball on the surface of the substrate; apical and basal ends

are not discernible. Cilia are absent and the surface is relatively smooth.

Pawn (Fig. 5). Six h after attachment a tiny bleb appears in the center of the disc and begins to elongate in an upright direction to form a shape that resembles the pawn of a chess set. The formation of the pawn from the disc stage requires 4 h. The original apical end of the planula forms the base of the pawn and the original basal end of the planula forms its head. The surface of the pawn is smooth and the first beginnings of a perisarc, an outer noncellular protective coating, are seen at the base.

Crown (Fig. 6). During the next 6 h the pawn elongates; the anterior head widens, forming a crown; and a sharp demarcation appears between the head and the stalk. This crown stage is formed 12 h after attachment, and it is during this stage that general features of the adult polyp begin to take shape: crown (future hypostome), stalk, base. The surface at this stage is smooth and covered with perisarc material.

Immature polyp (Fig. 7). After 2 h, 14 h after attachment, an immature polyp is formed. Its head region consists of a hypostome, a conical mound bearing the mouth at its tip, and a ring of forming filiform tentacles. These tentacles arise as tiny evaginations of the body wall at the base of the crown and lengthen to achieve the adult tentacle morphology. A clear division between the polyp head and the narrowing stalk is evident. The stalk connects the head to an enlarging base. The surface of the immature polyp below the region of the head is covered with perisarc.

Primary polyp (Figs. 8,9). Within 4 h, 18 h after attachment, a primary polyp is formed. A row of long, filiform tentacles and a new row of short, evaginating capitate tentacles, just above the filiform tentacles, characterize the crown region, constituting the fully formed adult hypostome (Fig. 9). A mouth is present at the very tip of the hypostome just above the whorl of capitate tentacles, a perisarc covers the stalk and basal region of the polyp, and stolon formation has begun in the basal region of the polyp (Fig. 8). These stolons produce additional polyps that remain attached to the original primary polyp, thus creating a colony.

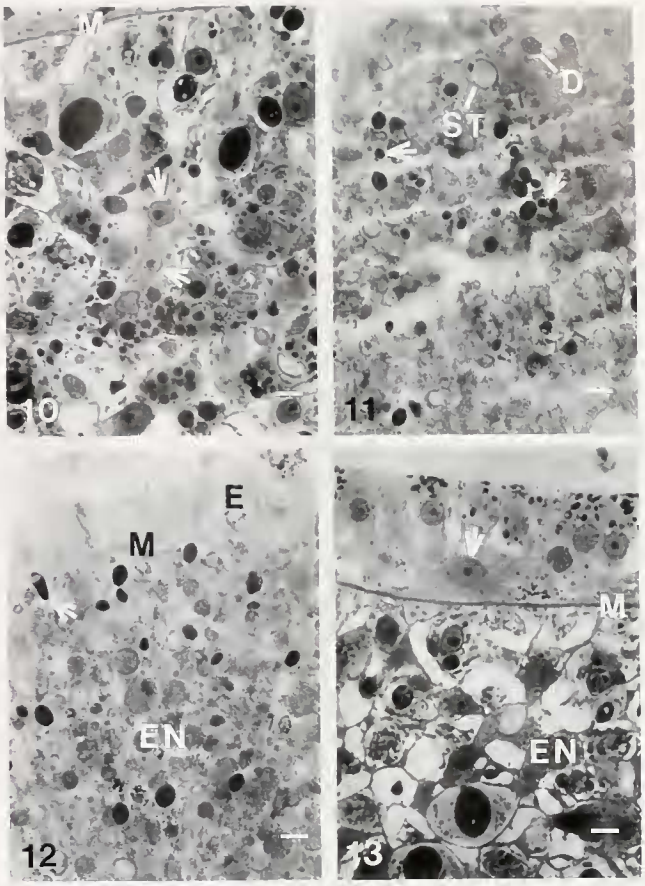


Figure 10. Interstitial cells (arrows) in the central endoderm of a mature planula. Each cell has a lightly stained cytoplasm plus a nucleus with a darkly stained nucleolus. M, mesoglea. Bar = 10 μm.

Figure 11. Nematoblasts with large dark capsules (D), small dark capsules (arrows), or large clear capsules (ST) in the endoderm of a mature planula. Bar = 10 μm.

Figure 12. Nematoblast with a bullet-shaped capsule (arrow) in the endoderm of a mature planula. Developing desmonemes (large dark capsules) and microbasic heterotrichous b-mastigophores (small dark capsules) are also seen. E, ectoderm; EN, endoderm; M, mesoglea. Bar = 10 μm.

Figure 13. Bipolar ganglion cell (arrow) in the ectoderm of a mature planula. The endoderm lacks neurons. EN, endoderm; M, mesoglea. Bar = 10 μm.

Interstitial stem cell system

Planulae of *Pennaria tiarella* contain a population of migratory stem cells, interstitial cells, that either divide to replenish the population or differentiate into two classes of somatic products: nematocytes (stinging cells) or ganglion cells (neurons). Early differentiating intermediates of the nematocyte lineage are called nematoblasts, and intermediates of the neural lineage are referred to as neuroblasts. Interstitial cells and their derivatives are easily identified in larval and adult tissue at the light microscopic level (Figs. 10–13). In the following sections we describe the behaviors of the interstitial cells, the nematoblasts and nematocytes, and the neuroblasts and ganglion cells during embryogenesis, in the metamorphosis-competent planula, during metamorphosis, and in the adult polyp.

Interstitial cells

Interstitial cells are small round cells measuring 7.5 μm in diameter (Fig. 10). They contain a centrally located nucleus with one or more darkly stained nucleoli.

These cells arise during gastrulation (8–10 h postfertilization), in the central core of the endoderm along the entire length of the young planula (Table I). They divide in the endoderm and by 13–14 h postfertilization begin to emigrate to the ectoderm, migrating as single cells through the interstitial spaces of the endoderm and through the mesoglea. By 15 h postfertilization, the interstitial cells reach the base of the ectoderm. Migration occurs along the entire apical-basal axis of the young planula. As planulae age, the numbers of interstitial cells in both the ectoderm and endoderm increase, and migration from the endoderm to the ectoderm continues along the entire planular axis. Thus the metamorphosis-competent planula has many interstitial cells at the base of its ectoderm and in the endoderm along its entire body axis (Table I).

During metamorphosis, as the apical pole of the planula attaches to a substrate and the basal end contracts towards the attached end, the interstitial cells located in the mid to basal regions of the larva move into the ectoderm and endoderm of the attachment region (Figs. 14–16; Table I). Their mechanism of movement is unknown but probably involves active cellular migration. The ectoderm and the endoderm of the remaining, still contracting basal portion of the attached larva become devoid of interstitial cells (Fig. 15; Table I). Once the disc stage is formed, interstitial cells fill both the ectoderm and the endoderm (Table I). As the center of the disc elongates in an upright direction, a pawn is produced (Fig. 17). The growing, apical upright tissue of the pawn, destined to form the head and stalk of the adult polyp, is devoid of interstitial cells (Fig. 17; Table I). These cells remain in the attached, now basal, end of the metamorphosing animal (Fig. 18); where the upright portion of the pawn connects to the basal disc is a sharp demarcation between presence of interstitial cells in the base and absence of these cells above the base. The distribution pattern of interstitial cells in the base of the pawn remains unchanged from that of the disc.

By the time the crown stage has formed, the interstitial cells have migrated out from the basal attachment site to populate the entire body axis of the animal (Fig. 19; Table I). In the attachment area (basal disc) a few interstitial cells are found in both the ectoderm and the endoderm. Along the body stalk, the region of the animal that connects the basal disc to the head, are scattered ectodermal interstitial cells and a few endodermal interstitial cells (Table I). The head of the crown stage has interstitial cells in both the ectoderm and the endoderm (Fig. 19; Table I). This same distribution pattern of interstitial cells is maintained in the immature polyp and in the adult primary polyp (Table I). In the primary polyp many interstitial cells are seen at the base of each filiform tentacle, but none are seen within the tentacles.

Nematoblasts and nematocytes

Nematoblasts, immature nematocytes, range from 10 to 12.5 μm in diameter and form distinctive dark-staining or light-staining capsules (Figs. 10–12); each capsule contains a nematocyst thread that may possess barbs and spines. Nematoblasts are found in both the ectoderm and the endoderm throughout embryogenesis, metamorphosis, and in the adult polyp. Once nematoblasts move to the outer surface of the ectoderm or project into a forming gastric cavity, they complete their differentiation and are considered functional nematocytes. A few nematoblasts with dark capsules are first detected in the apical endoderm of the young 10-h planula (Table I). Migration of the nematoblasts begins by 13 h postfertilization, and these cells are the first of the interstitial cell system to appear in the ectoderm (by 14 h postfertilization) of the planula. Nematoblasts in the apical endoderm migrate as single cells into the apical ectoderm; they do not divide and syncytial clusters of nematoblasts are not observed at any stage of the life cycle. As planulae mature the nematoblasts increase in number in both the ectoderm and the endoderm and are largely confined to the apical two-thirds of the planular axis (Table I). At least four types of capsules have been observed in the mature planula (Figs. 10–12): a large clear capsule (stenoteles), a large dark capsule (desmonemes), a small dark capsule (microbasic heterotrichous b-mastigophores), and a metachromatic bullet-shaped capsule (microbasic heterotrichous b-mastigophores with inclusions). Stenoteles and desmonemes predominate; only a few microbasic heterotrichous b-mastigophores with inclusions are seen.

Fully differentiated nematocytes are found only at the surface of the mature planula, the majority in an area extending from the apical end of the planula to the mid planula (Table I); only a few nematocytes are found at the surface in the basal (posterior) region. Fully differentiated nematocytes of planulae contain either a large clear capsule (stenoteles), a large dark capsule (desmonemes) or a bullet-shaped capsule (microbasic heterotrichous b-mastigophores with inclusions); no fully differentiated nematocytes housing the small dark capsules (microbasic heterotrichous b-mastigophores) are found in the planula.

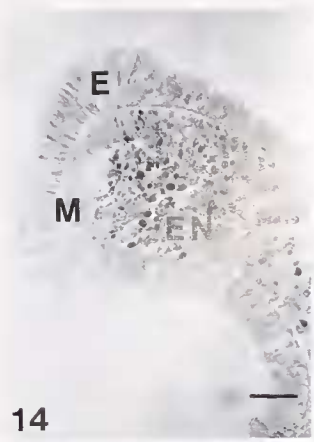
As planulae attach to substrates, all nematoblasts move into the ectoderm and endoderm of the apical attachment region (Figs. 14 and 15). Nematocytes are confined to the outer surface of the ectoderm of the attachment area. Hence, the contracting basal portion of the attached larva is devoid of nematoblasts and nematocytes in both the ectoderm and the endoderm (Table I). All four types of nematoblast capsules are detected in the ectoderm and endoderm of the attachment area. The bulk of these cells are differentiating stenoteles, desmonemes, and microbasic

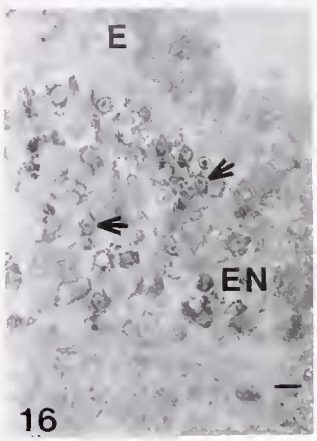
Table I

Distribution of the interstitial cell system during development of Pennaria tiarella

Stage	Interstitial cells	Nematoblasts	Nematocytes	Ganglion cells
10-Hour Planula				
Apical Ectoderm	—	—	—	—
Mid Ectoderm	—	—	—	—
Basal Ectoderm	—	—	—	—
Apical Endoderm	+	+	—	—
Mid Endoderm	+	—	—	—
Basal Endoderm	+	—	—	—
48-Hour Planula				
Apical Ectoderm	++	++	++	++
Mid Ectoderm	++	++	++	++
Basal Ectoderm	++	+	+	++
Apical Endoderm	++	++	—	—
Mid Endoderm	++	++	—	—
Basal Endoderm	++	+	—	—
Attaching Planula				
Apical Ectoderm (Attachment site)	++	++	+	—
Mid Ectoderm	—	—	—	—
Basal Ectoderm	—	—	—	—
Apical Endoderm	++	++	—	—
Mid Endoderm	—	—	—	—
Basal Endoderm	—	—	—	—
Disc Stage				
Ectoderm	++	++	+	—
Endoderm	++	++	—	—
Pawn Stage				
Apical Ectoderm (Head and Stalk)	—	—	—	—
Basal Ectoderm (Foot)	++	++	+	+
Apical Endoderm (Head and Stalk)	—	—	—	—
Basal Endoderm (Foot)	++	++	—	—
Crown Stage				
Apical Ectoderm (Head)	+	+	+	—
Mid Ectoderm (Stalk)	+	+	+	+
Basal Ectoderm (Foot)	+	++	+	++
Apical Endoderm (Head)	+	+	—	—
Mid Endoderm (Stalk)	+	+	—	—
Basal Endoderm (Foot)	+	+	—	—
Immature Polyp				
Apical Ectoderm (Head)	+	+	+	++
Apical Ectoderm (Tentacle)	—	+	++	++
Mid Ectoderm (Stalk)	+	+	+	+
Basal Ectoderm (Foot)	+	++	+	++
Apical Endoderm (Head)	+	+	—	—
Apical Endoderm (Tentacle)	—	—	—	—
Mid Endoderm (Stalk)	+	+	—	—
Basal Endoderm (Foot)	+	+	—	—
Primary Polyp				
Apical Ectoderm (Head)	+	+	+	++
Apical Ectoderm (Tentacle)	—	+	++	++
Mid Ectoderm (Stalk)	+	+	+	+
Basal Ectoderm (Foot)	+	++	+	++
Apical Endoderm (Head)	+	+	—	—
Apical Endoderm (Tentacle)	—	—	—	—
Mid Endoderm (Stalk)	+	+	—	—
Basal Endoderm (Foot)	+	+	—	—

Table Key: ++ = Abundant to moderate in number. + = A few present. — = Absent.





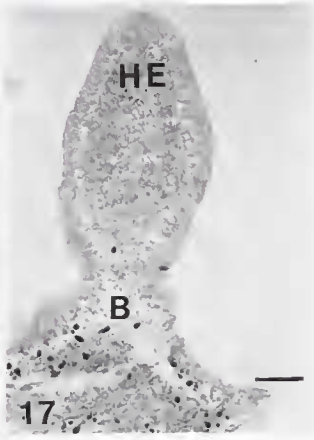


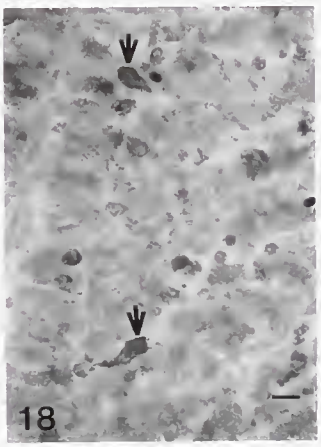
Figure 14. Apical region of an attaching planula. The interstitial cell system has moved into the attachment area; note the large number of dark nematoblast capsules in this region. E, ectoderm; EN, endoderm; M, mesoglea. Bar = 50 μm.

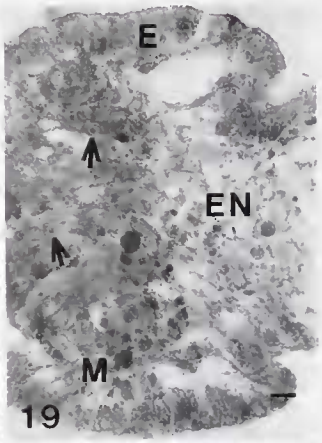
Figure 15. Mid to basal region of an attaching planula. Note the absence of the interstitial cell system in the basal portion (B) of the animal. E, ectoderm, EN, endoderm; M, mesoglea. Bar = 50 μm.

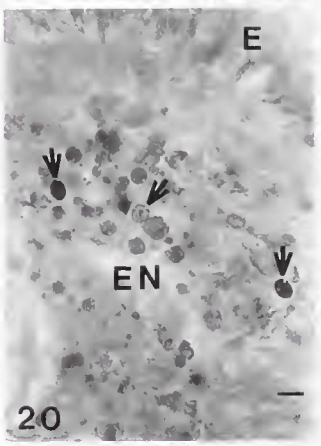
Figure 16. Interstitial cells (arrows) in the attachment region of a metamorphosing planula. E, ectoderm; EN, endoderm. Bar = 10 μm.

Figure 17. Pawn. The apical head region (HE) is devoid of the interstitial cell system. These cells remain in the basal region (B) of the pawn. Dark nematoblast capsules are abundant in the base. Bar = 50 μm.

heterotrichous b-mastigophores; only a few developing microbasic heterotrichous b-mastigophores with inclusions are found. Once the disc stage is reached, the four types of nematoblasts fill the ectoderm and endoderm (Fig. 20); a few nematocytes (stenoteles and desmonemes) are observed around the ectodermal surface of the disc (Table I). As the center of the disc elongates to form the pawn, the nematoblasts and nematocytes remain in the attachment disc (Figs. 17 and 21). Thus, the upright growing tissue is devoid of nematoblasts and nematocytes. These cells are confined to the substrate-attached basal disc region of the metamorphosing animal and resemble the pattern described for the disc stage (Table I). By the time the crown stage has formed, the interstitial cell system has migrated from the basal attachment site to populate the entire body axis of the animal. Nematoblasts are the first of the line to appear apically (Figs. 22–24), and distinct patterns of nematoblast and nematocyte distribution are observed. In the ectoderm of the attachment area are all four types of nematoblasts and two types of nematocytes (stenoteles and desmonemes). On either side of the basal disc just above the substrate attachment zone, the perisarc is connected to the basal disc and lower body column. In these regions of perisarc attachment to the ectoderm, the ectoderm has an abundance of nematoblasts (desmonemes and stenoteles) (Fig. 22). Along the body stalk of the crown in the ectoderm are the four types of







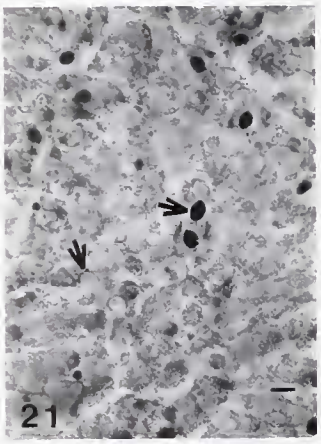


Figure 18. Interstitial cells (arrows) in the endoderm at the base of the pawn. These cells are migrating as indicated by the presence of a single filopodial-like extension. Bar = 10 μm.

Figure 19. Head region of the crown stage. Interstitial cells (arrows) are detected; however, ganglion cells are absent in this area. E, ectoderm; EN, endoderm; M, mesoglea. Bar = 10 μm.

Figure 20. Disc stage. Nematoblasts (arrows) are abundant in the endoderm; ganglion cells are absent. E, ectoderm; EN, endoderm. Bar = 10 μm.

Figure 21. Nematoblasts (arrows) in the endoderm at the base of the pawn. Bar = 10 μm.

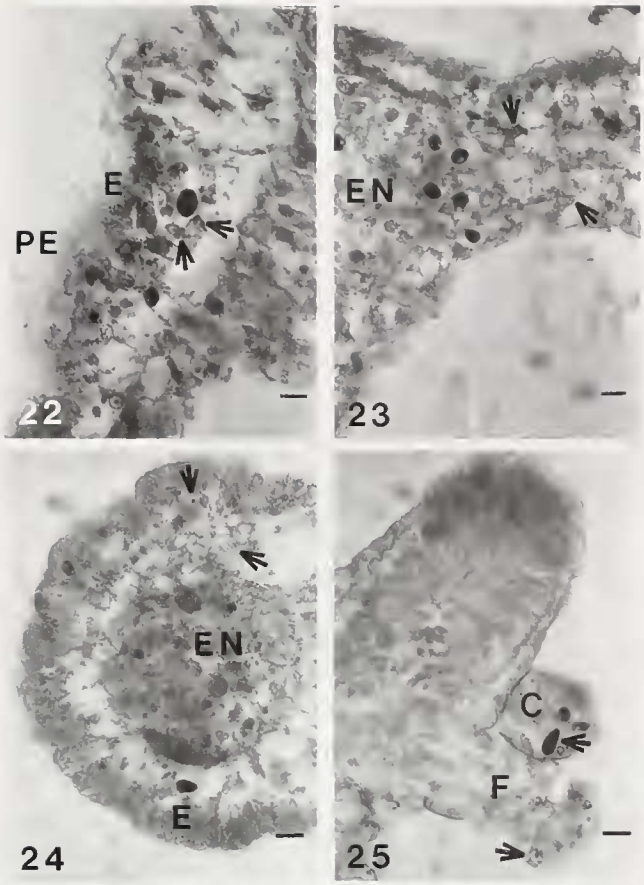


Figure 22. Crown stage. The perisarc (PE) is attached to the ectoderm (E) just above the foot region of the forming polyp. This region is rich in ganglion cells (arrows) and nematoblasts. Bar = 10 μm.

Figure 23. Stalk region of the crown stage showing multipolar ganglion cells (arrows) and nematoblasts. EN, endoderm. Bar = 10 μm.

Figure 24. Stenoteles (arrows) and a nematoblast with a dark bullet-shaped capsule in the head region of the crown stage. E, ectoderm; EN, endoderm. Bar = 10 μm.

Figure 25. Head region of a primary polyp showing nematoblasts (arrows) in a capitate tentacle (C) and a filiform tentacle (F). Bar = 10 μm.

nematoblasts and at the surface a few nematocytes (stenoteles and desmonemes). The endoderm of the stalk has a few nematoblasts (desmonemes and stenoteles) (Fig. 23). In the head of the crown in both the ectoderm and the endoderm are three types of nematoblasts: desmonemes, stenoteles, and microbasic heterotrichous b-mastigophores with inclusions (Fig. 24). Three kinds of nematocytes, the same varieties as the head nematoblasts, project from the ectodermal surface of the head. Prior to the crown stage, the nematoblasts with bullet-shaped capsules (microbasic heterotrichous b-mastigophores with inclusions) are seen sparingly along the whole body axis of metamorphosing animals; however, by the crown stage many nematoblasts of this type have accumulated in the head.

As the crown stage transforms into the immature polyp, the ectoderm of the forming filiform tentacles becomes filled with three types of nematoblasts and nematocytes: stenoteles, desmonemes, and microbasic heterotrichous b-mastigophores with inclusions. Other than this change, the distribution pattern of the nematoblasts and nematocytes is the same as seen in the crown stage.

As immature polyps form primary adult polyps, a second group of short tentacles, the capitate tentacles, appears just above the whorl of filiform tentacles (Fig. 25). These capitate tentacles are populated with the same three types of nematoblasts and nematocytes that occupy the filiform tentacles (Fig. 25). Along the body column mature nematocytes with small dark capsules (microbasic heterotrichous b-mastigophores) are visible. Other than these changes, the nematoblast and nematocyte system of the primary polyp resembles that of the immature polyp. Thus in the primary polyp the concentration of nematoblasts and nematocytes is high in the head and in the foot and scattered in the stalk. The distribution pattern of nematocytes is specific: in the head are stenoteles, desmonemes, and microbasic heterotrichous b-mastigophores with inclusions; along the body column are stenoteles, desmonemes, and microbasic heterotrichous b-mastigophores; and in the foot are desmonemes and stenoteles.

Neuroblasts and ganglion cells

Differentiating neuroblasts are detected as early as 16–20 h postfertilization in both the ectoderm and endoderm of the planula (Brumwell and Martin, 1996). These first neuroblasts arise in the apical region of the larva; shortly thereafter they are found along the entire length of the planula. Neuroblasts are small round cells, similar in size to interstitial cells, that contain cytoplasm rich in neurosecretory vesicles (Brumwell and Martin, 1996). These differentiating intermediates migrate as single cells from the endoderm to the base of the ectoderm; neuroblasts are positioned closer to the mesoglea than are the interstitial cells of the ectoderm. Neuroblasts seemingly emigrate in a straight path from the endoderm to the ectoderm; there is no evidence that they migrate in an apical or basal direction in the larva. Once neuroblasts reach the basal ectoderm they stop moving and complete their differentiation by extending neural processes. These processes are filled with neural vesicles and form an extensive neural plexus of transversely and longitudinally oriented processes throughout the length of the planula. As the planula ages additional ganglion cells differentiate and incorporate into the larval network. Fully differentiated larval ganglion cells are 5 μm in diameter, bipolar, spindle-shaped, and positioned in the ectoderm just above the mesoglea along the entire apical, basal axis of the planula (Fig. 13; Table I).

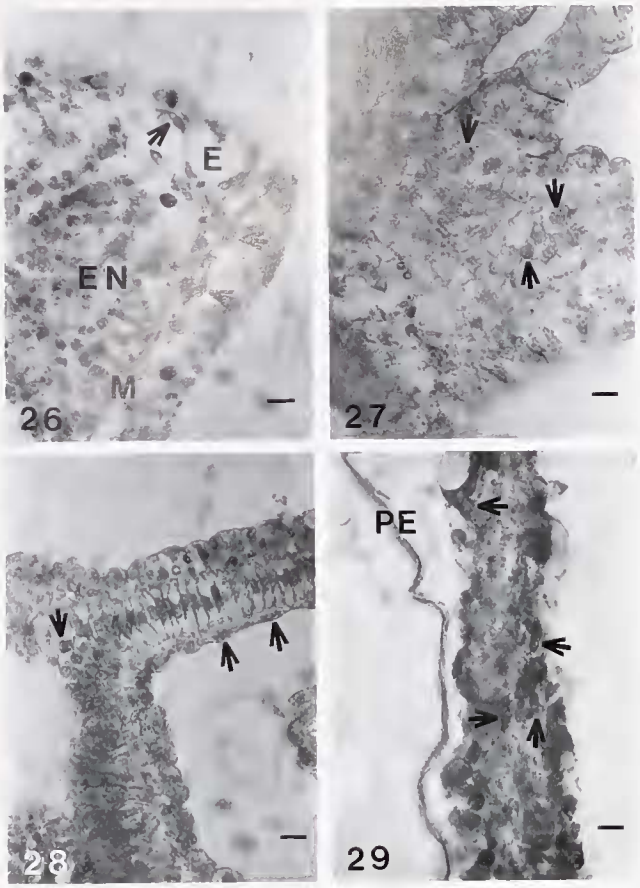


Figure 26. Neurons (arrows) and nematoblasts with dark capsules in the basal disc of the crown stage. E, ectoderm; EN, endoderm; M, mesoglea. Bar = 10 μm.

Figure 27. Ganglion cells (arrows) in the head region of a primary polyp. Bar = 10 μm.

Figure 28. Filiform tentacle of a primary polyp. Note the abundance of neurons (arrows) at the base of the tentacle and along its length. Bar = 10 μm.

Figure 29. Stalk of a primary polyp. Note the ganglion cells (arrows) in the ectoderm. PE, perisarc. Bar = 10 μm.

During attachment and the early stages of metamorphosis, the larval ganglion cells disappear; by the disc stage they are gone (Table I). In the pawn a few ganglion cells differentiate in the basal disc (Table I). These neurons are found in the ectoderm just above the mesoglea; they are triangular or star-shaped and are multipolar. These neurons have smaller cell bodies and thinner processes than did the planular ganglion cells. By the crown stage ganglion cells are found in the ectoderm of the basal disc and the body stalk (Table I). The basal disc contains a large number of ectodermal bipolar and multipolar ganglion cells (Fig. 26), and multipolar ganglion cells are abundant in the region of perisarc attachment to the basal disc and lower body column (Fig. 22). Along the body stalk in the ectoderm are at least three types of ganglion cells: triangular-, star- or spindle-shaped (Fig. 23). Ganglion cells are not detected in the head of the crown stage. As the crown stage develops into the immature polyp, many ganglion cells (bipolar, multipolar) appear in the head ectoderm and tentacles (Table I). By the primary polyp stage, the head of the animal is enriched in ganglion cells, especially around the mouth (Fig. 27). Ganglion cells are found at the base of each filiform tentacle and in the ectoderm along the lengths of the tentacles (Fig. 28). In the ectoderm of the body stalk are scattered ganglion cells (Fig. 29), and the distribution pattern in the basal disc mimics that of the immature polyp (Table I). Thus in the primary polyp there is a high concentration of ganglion cells in the ectoderm of the head and basal disc and some scattered ganglion cells in the ectoderm of the body column (Table I). The ganglion cells of the polyp are three types: spindle-shaped bipolar, star-shaped multipolar, and triangular-shaped multipolar.

Discussion

The transformation of the cnidarian planula larva into the adult phenotype is rapid, taking only 18–20 h in the hydrozoan *Pennaria tiarella*, and is characterized by general body reorganization and modification of the stem cell system. During metamorphosis the hydrozoan planula ceases swimming, loses its cilia, and attaches to the substrate by its apical (aboral) pole. Both glandular secretions and nematocytes may be used for securing planulae to a substrate (Martin *et al.*, 1983). Shortly after attachment the basal (oral) end of the larva contracts down towards the apical pole until it disappears into the attached pole. A tiny circular disc is formed. Next, a tiny bleb appears in the center of the disc and begins to elongate in an upright direction forming a pawn shape. Three distinct regions of the pawn are evident: an apical head, a mid-stalk region, and a basal disc. The pawn grows and reshapes to produce a crown stage, in which general features of the adult polyp begin to take shape: head, stalk, and base, with a clear separation between the head and the stalk. Tentacles evaginate from the head region and a mouth breaks through at the tip of the head, producing an immature polyp. This stage becomes a primary polyp when a row of long filiform tentacles and a row of short capitate tentacles adorn the head. A mouth is present at the very tip of the head just above the whorl of capitate tentacles, and a perisarc covers the stalk and basal region of the polyp. To form a colony, the polyp extends stolons from the base and asexually buds additional polyps, all of which remain connected together.

Four major changes occur in the interstitial cell system during metamorphosis: (1) the distribution pattern of the cells along the body axis changes, (2) certain larval derivatives disappear, (3) new types of derivatives differenti-

ate, and (4) migration patterns become more complex. Comparisons of the distribution patterns of the interstitial cell system in the planula and in the adult clearly show that these cells undergo dramatic reorganization during metamorphosis. This is not surprising given the fact that the entire polarity of the animal changes as a planula forms an adult: the apical end of the planula becomes the basal end of the polyp, and the mid-to-basal end of the planula forms the stalk and hypostome of the adult. In the planula, interstitial cells and ganglion cells are found along the entire apical-basal axis, whereas the majority of the nematoblasts and nematocytes are confined to the apical two-thirds of the planula. During metamorphosis this whole larval pattern is lost; from the disc stage onward a new adult pattern of the interstitial cell system is established. By the time the primary polyp has formed interstitial cells, nematoblasts and ganglion cells are found along the entire body axis; the concentration of ganglion cells and nematoblasts is high in the foot; the concentration of ganglion cells is high in the hypostome; the concentration of ganglion cells and nematocytes is high in the tentacles; and specific types of nematocytes are confined to distinct regions of the polyp.

In the mature planula two major kinds of derivatives differentiate from interstitial cells: a single type of bipolar ganglion cell and four types of nematoblasts. Of the latter only three types (desmonemes, stenoteles, and microbasic heterotrichous b-mastigophores with inclusions) complete differentiation in the planula; only a few nematocytes with bullet-shaped capsules (microbasic heterotrichous b-mastigophores with inclusions) were ever found in the planula. The other variety (microbasic heterotrichous b-mastigophores) begins development in the planula but completes differentiation only during metamorphosis. The larval bipolar ganglion cells disappear during planular attachment, suggesting that these neurons play a role in the attachment of the planula to the substrate. A subpopulation of these larval ganglion cells contain a RFamide-like peptide that may be used to initiate metamorphosis, to propagate it, or both (Brumwell and Martin, 1996). In fact, several investigators have previously proposed that the larval neurons are involved in propagating the metamorphic signal to other cells in the planula (Martin and Thomas, 1981b; Thomas *et al.*, 1987; Plickert, 1988; Leitz *et al.*, 1994; Leitz and Lay, 1995; Gajewski *et al.*, 1996; Brumwell and Martin, 1996).

In the adult polyp two types of somatic derivatives arise from interstitial cells: ganglion cells, of which there are at least three varieties, and nematocytes, of which there are four kinds. These new neurons begin to appear at the pawn stage and are found throughout the remaining phases of metamorphosis. Three types of nematocytes (desmonemes, stenoteles, and microbasic heterotrichous b-mastigophores with inclusions) first appear in the planula and are found throughout metamorphosis, whereas microbasic heterotrichous b-mastigophores, which begin but never complete differentiation in the planula, appear at the ectodermal surface between the crown stage and the polyp stage. Furthermore, by the polyp stage, fully differentiated microbasic heterotrichous b-mastigophores with inclusions have increased in number and are abundant in the head. The appearance of new somatic stem cell derivatives during metamorphosis, notably the three kinds of ganglion cells, indicates that the interstitial cells have a greater differentiative potential than demonstrated in the planula.

The migration patterns of the interstitial cell system are more complex in the adult than in the planula. In the planula the interstitial cells, nematoblasts, and neuroblasts emigrate from the endoderm to the ectoderm. There is no evidence that once in the ectoderm, these cells migrate in an apical-basal direction (Martin and Archer, 1986)—they appear to stay close to the site at which they entered the ectoderm. During metamorphosis this changes: interstitial cells, nematoblasts, and possibly neuroblasts migrate apically in large numbers. As the pawn stage arises from the disc, the emerging stalk and head of the pawn form and initially are completely devoid of a stem cell system. Between the pawn stage and the crown stage, the stem cells migrate apically in large numbers and populate the stalk and head of the crown. The first cells of the stem cell line to appear anteriorly are nematoblasts, followed by interstitial cells and lastly by ganglion cells. Interestingly, this is the order of appearance of interstitial cell types in the ectoderm of the planula shortly after larval interstitial cell migration begins (Martin and Archer, 1986). The introduction of the stem cell system into the forming apical region of the crown may be essential for shaping of the hypostome and for tentacle formation. Coincidentally, it is during the crown stage that general features of the adult hypostome begin to take shape and the stem cell system first appears in the apical end of the metamorphosing animal. Because interstitial cells and nematoblasts are found in the endoderm of adult polyps, it is possible that these cells can move from the endoderm to the ectoderm. Thus in the adult, multiple directions of migration are probable. Furthermore, since specific types of cells accumulate in specific regions of the polyp—for example, neurons are abundant in the head and the foot—some sort of directed migration must occur during metamorphosis.

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